NOVEL AND CONVENTIONAL CONTROL OF *LINARIA GENISTIFOLIA SSP. DALMATICA* IN A SEMI-ARID GRASSLAND OF BRITISH COLUMBIA'S SOUTHERN INTERIOR

By

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// Abstract

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Dalmatian toadflax (*Linaria genistifolia ssp. Dalmatica*) is a major concern in disturbed areas of British Columbia's interior due to its pronounced ability to displace native vegetation. To investigate the most appropriate control method, including efficiency, a study was established in Kenna Cartwright Nature Park, Kamloops, BC in 2016. Manual removal and three herbicide treatments were compared by collecting live and dead stem count in 6m by 6m replicates (n=30) during baseline, post-treatment and end-of-growing season assessments. Tordon 22K was applied at 213 g a.e. ha^{-1} picloram mixed with Agral 90 surfactant (0.025% by volume) using broadcast spraying, spot spraying and hand wicking (August 23rd). Treatment had a significant effect; broadcast and spot spraying provided the highest overall success resulting in mean stem counts of 1.7 ± 1.07 and 6.33 ± 2.67 at the end of the growing season, respectively. Wicking offered the same overall success as spot spraying in reducing total live stems. However, wicking contained 61% of the live stems at the end of the growing season, relative to baseline, whereas spot spraying only contained 31%. Broadcast spraying offered the greatest success in which survivorship was 13%. Only broadcast and spot spraying provide statistically significant reduction in vegetative stems from baseline to end-of-season; broadcast spraying contained zero stems in this life stage. Despite eliminating all stems at time of treatment, manual removal experienced high regrowth which resulted in statistical similarity to the control and appeared to encourage vegetative regrowth. Nearly all of the regrowth experienced in the control, manual removal, spot spraying and wicking at the end of the growing season were rosettes. Seedlings did not greatly contribute to reproduction in any treatments and were absent from broadcast spraying. For high density sites (>31 stems/m^2), broadcast spraying offers the greatest chance of success based on both success and efficiency. Low to medium density sites can be controlled by spot spraying, however the lower residual soil activity must be considered. Ultimately, the effect on the native plant community must be evaluated when selecting one of the recommended treatment methods and future research should focus on tracking the communities through time.

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A great deal of gratitude goes to the City of Kamloops for funding this research and trusting this investigation with me in such a valuable nature park. A special thanks to Kirsten Wourms for facilitating this opportunity and having such encouraging excitement for the project. Thanks to Adam Fehr for helping me implement the treatments and providing valuable logistical advice. I would also like to thank Andrea Seager and Chantelle Gervan for helping me establish the experiment and collect data.

Dedication

This thesis is in memory of my late brother and dedicated to my mother, father and brother for supporting my move across the country to pursue this degree and for encouraging me to chase all opportunities, no matter where they take me.

Introduction

Invasive alien species play a significant role in the fate of native ecosystems, specifically whether our environment will continue to prosper and retain the same resilience that has encouraged diverse landscapes across Canada (Vitousek et al. 1997; EC 2004; Winstral and Marks 2014). Globally, they are considered one of the most significant threats to biodiversity by the World Conservation Union, second only to habitat loss, and are a critical priority for the government of Canada (EC 2004; GOC 2016). Invasive species are those that have become established outside of their natural past or present distribution, largely through intentional or accidental human introduction, and threaten biological diversity (Vitousek et al. 1997; CBD 2008; Pyšek and Richardson 2010; IUCN). Although typically only 5% to 20% of introduced species spread and impact the destination ecosystem in this manner, the negative outcomes can be extensive due to their pronounced ability to out-compete native organisms (CBD 2008; Pyšek and Richardson 2010; IUCN 2017). Additionally, the sheer number of introductions has a cumulative impact on native biodiversity, as has been observed during the 76% increase in invasive species in Europe since 1970 (Butchart et al. 2010). This large increase is common across the globe; in British Columbia there are currently 175 invasive plants (ERBC 2015). The influence of these species transcends biological kingdoms. For example, about 400 of 958 threatened or endangered species are at risk due to nonindigenous species in the United States (Wilcove et al. 1998 as cited in Pimentel et al. 2005)

Economic and Environmental Impacts of Invasive Species

The total annual economic burden of invasive species in Canada is nearly $30 billion, $2.2 billion of which is directly related to invasive plants (CFIA 2014). The high cost is due to a combination of chemical and mechanical control, loss of crop productivity and increased need for specialized equipment and personnel (EC 2004; GOC 2016). In 2008, total economic burden for British Columbia was $65 million and without continued intervention, invasive species are projected to cost B.C. $139 million a year by 2020 (IPCBC 2008). Today these species continue to place a significant financial burden on landowners and tax payers throughout the province, costing the agricultural industry alone $50 million each year (ISCBC 2017). However, more concerning is the threat to society far beyond the direct revenue and mitigation costs. Alien species have the potential to reduce water quality, eliminate traditional food and medicinal plants, degrade human health and reduce recreational opportunities (Vitousek et al. 1997; Pejchar and Mooney 2009; Pyšek and Richardson 2010; ISCBC 2017).

With the potential to influence energy and nutrient cycling, invasive species interrupt the traditional synergy of wildlands and place a burden on community structure that has been consistently compounded by human development and environmental manipulation (Pyšek and Richardson 2010). As a result of these negative impacts, invasive species have been identified as a threat by local organizations such as the Thompson Nicola Regional District and the City of Kamloops (City of Kamloops no date; TNRD no date). Local efforts for controlling invasive plants are in-line with the four stages through which the Government of Canada intends to respond to invasive alien species, prevention of new invasions, early detection of new invaders, rapid response to new invaders, and management of established and spreading invaders (EC 2004; Pyšek and Richardson 2010). Although many institutions have played a role in these four responses, historically the Canadian Food and Drug Agency has spear-headed prevention and provincial bodies such as the B.C. Early Detection Rapid Response coordinate efforts on the second and third stages (EC 2004; Pyšek and Richardson 2010; BCIMISWG 2014). On-the-ground invasive plant management has typically been implemented by municipal government and private individuals (Pyšek and Richardson 2010). It is in the final response, management, that this study intends to provide a greater understanding of the potential control methods for the invasive alien, Dalmatian toadflax (*Linaria genistifolia ssp. dalmatica*; henceforth toadflax).

Dalmatian Toadflax Life History and Response to Disturbance

One of the most formidable species threatening our region's natural areas is Dalmatian toadflax, an invasive plant that is considered provincially noxious in British Columbia (Scott 1999; Ralph et al. 2014; TNRD no date; City of Kamloops no date). This species is native to the Mediterranean region of Dalmatian and was brought to North America as an ornamental species in 1874 (Alex 1962 as cited in Ogden and Renz 2005). When in dense stands, this plan reduces wildlife habitat quality and competitively excludes native flora (Lajeunesse 1999 as cited in Kyser and DiTomaso 2013). Compounding this issue is the self-incompatible nature of toadflax which is speculated to have lead to the high level of genetic variability within the species (Docherty 1982 as cited in Kyser and DiTomaso 2013). This plant is characterized by pale green waxy leaves clasping the stem and conspicuous "snapdragon-like" yellow flowers (2.5 cm to 4 cm long) (Ralph et al. 2014). Toadflax stands 1.2 m tall and produces a creeping perennial root system (Scott 1999; Ralph et al. 2014). The species is now an aggressive invader, occurring from sea level to 2800 m in B.C. (Scott 1999; Jacobs 2006; Ralph et al. 2014).

Understanding the biology of toadflax allows for the appropriate control method to be utilized at the appropriate stage in the plant's life cycle. This species has several morphological adaptations that allow it to prosper in stressful environments. These are expressed in the dynamic relationship between toadflax's phenology, competing vegetation and climate. Reproductive potential is perhaps the greatest of such advantages. Dalmatian toadflax is a prolific seed producer with rapid spring germination, allowing it to gain an advantage on native species (Scott 1999). This is, in part, due to it's ability to sprout from adventitious root buds as early as 14-21 days after germination and to produce up to 500,000 seeds per plant on low competition sites, which may remain viable for ten years (Robocker 1968; Robocker 1970; Scott 1999; Jacobs 2006). Dalmatian toadflax is able to out-compete neighbouring vegetation under diverse disturbance regimes due to these life history attributes.

Toadflax is an effective pioneer following fire due to its extensive perennial sprouting root system (Zouhar 2003; Jacobs 2006). Fire also has the potential to increase seed production and is therefore not a recommended treatment option (Zouhar 2003; Jacobs 2006). There is a high potential for post fire colonization in semi-arid grasslands due to toadflax's proliferation on dry areas with low competition (Zouhar 2003). This has lead to several cases where the presence of toadflax was not known before fire but there was rapid population growth post-fire (Zouhar 2003). Due to these traits, early detection is key in preventing toadflax from establishing in post-burn areas. This is the least costly and most effective control measure, especially when toadflax cover is greater than twenty percent (Goodwin and Sheley 2001; Clark 2003).

Biological control of Dalmatian toadflax has been underway in Canada and the United States since the 1960s (Kyser and DiTomaso 2013). However, considering the wide range of environmental conditions this plant tolerates, the seven biocontrol species that have been released have each experienced a wide range of establishment and control success (Kyser and DiTomaso 2013). The high genetic variability of the plant also negatively impacts biological control success and has lead to a lack of long-term stand reductions (Jamieson and Bowers 2010 as cited in Kyser and DiTomaso 2013; Kyser and DiTomaso 2013). One of the most successful biological agents for Dalmatian toadflax is the stem dwelling weevil, *Mecinus janthinus*, which can exert top-down effects on the plant during high densities and attack rates when stem diameter is large enough for it oviposit and pupate (Jamieson et al. 2012). Other common agents include beetles, moths and weevils (Kyser and DiTomaso 2013).

Manual and mechanical removal of toadflax is most successful when the population is small and easy to isolate (Scott 1999). Hand-pulling is a viable option in moist sandy soils. This method is recommended in wilderness areas due to it's low disturbance, relative to cultivation, but could require ten years of repeated treatment to eradicate a population (USDA 2012). To ensure maximum effectiveness, the entire root must be removed as toadflax has the ability to reproduce from root fragments as small as 1 cm (Bakshi and Coupland 1960 as cited in Ogden and Renz 2005; USDA 2012; Lajeunesse et al. 1993, Jacobs and Sing 2006 as cited in Kyser and DiTomaso 2013; Kyser and DiTomaso 2013; Wilson et al. 2005). Another alternative is cutting, which can be effective in specific situations (Scott 1999; USDA Forest Service 2012). However, since above-ground removal of biomass can stimulate adventitious root buds, cutting must be timed appropriately considering the plant's extensive carbohydrate stores (Scott 1999; Sheley and Clark 1999; Zouhar 2003; Bakshi and Coupland 1960 as cited in Ogden and Renz 2005; USDA 2012). The remaining mechanical control methods, mowing and tillage, are not recommended for toadflax because of its ability to vigorous re-sprout from its roots (Wilson et al. 2005).

Herbicide treatment is an important component of toadflax control, but requires high rates of application and does not result in long-term control of the species when used independently of other control measures (Scott 1999; USDA 2012; Kyser and DiTomaso 2013). An important consideration in chemical treatment is whether seeding will be required; this is not necessarily required in areas of high native grass abundance and cover (USDA 2012). In wilderness or natural areas, the use of backpack or hand-held herbicide applicators is preferred, both due to lower potential for non-target mortality and regulatory framework (USDA 2012). Picloram is commonly cited as one of the most successful herbicides for Dalmatian toadflax control because it s translocated through the plant and causes growth abnormalities (Robocker 1968; Duncan 1999 as cited in Kyser and DiTomaso 2013; Kyser and DiTomaso 2013; KCE 2014; USDA 2012). As a commercial product Tordon 22K from DOW AgroSciences offers picloram at 0.24% concentration and is a recommended off-the-shelf management tool because it provides the necessary soil-residual activity of approximately three years (Ogden and Renz 2005; DowAS 2009; USDA 2012; Kyser and DiTomaso 2013). Tordon 22K is selective at the broadleaf level. There is contradicting literature concerning which time of application of picloram is most appropriate, all of which are referenced by management agencies throughout North America. Jacobs and Sheley (2005) and Jacobs (2006) suggest spring application before toadflax develops its waxy cuticle, where as Robocker (1968) suggests fall application in granular form to create soil residual herbicide. Additionally, Kadrmas and Johnson (2002), Ogden and Renz (2005) and DiTomaso et al. (2013) recommend application at any stage of growth is appropriate when considering that picloram can has soil residual properties, greater success following first frosts, and is applied to target rapidly growing stages. The active ingredient concentration (a.e.) when using picloram to control toadflax varies depending on the application method, soil pH, soil texture, interspecific competition and timing of application (Robocker et al. 1972; Jacobs and Sheley 2005). The range is wide, from 0.14 kg a.e. ha^{-1} to 2.52 kg a.e. ha^{-1} (Robocker 1968; Robocker et al. 1972; Ferrell and Whitson 1989; Denny 2003; Jacobs and Sheley 2005; Ogden and Renz 2005; Jacobs 2006; DiTomaso et al. 2013). To increase the amount of picloram delivered to Dalmatian toadflax, surfactants are recommended at 0.25% to 0.5% by volume to counteract the waxy cuticle on the leaf which easily sheds liquid and may interfere with herbicide uptake (USDA 2012; Lajeunesse et al. 1993, Sing 2006 as cited in Kyser and DiTomaso 2013; DiTomaso et al. 2013). Considering the variability in treatment methodology and constituents, toadflax is most effectively controlled by combining treatments that act upon multiple life stages of the plant (Scott 1999). With all treatment methods, especially herbicide, the effect on non-target species must be considered.

Knowledge Gaps

Society is demanding less use of chemicals in our natural environment under the perception that it will have an immediate positive effect on biodiversity. However, invasive species will continue to have the opportunity to proliferate until integrated management strategies are perfected. To reduce the spread of invasive species in the interim, it is important to identify effective low-impact control methods. This research will contribute to the conservation of natural ecosystems through an increased understanding of the herbicide and manual eradication possibilities for Dalmatian toadflax. Reduced use of herbicide is a priority for many jurisdictions throughout Canada, including the City of Kamloops, and is critically important in protected areas, such as Kenna Cartwright Nature Park. Therefore, it is also important to consider the efficiency of treatment options while maintaining high eradication rates on an operational level.

Objectives

The purpose of this research was to determine the most effective control method for Dalmatian toadflax in a semi-arid grassland ecosystem that experiences frequent anthropogenic use. Considering the limitations of previous research, the eradication success of manual removal, broadcast spraying, spot spraying, and wicking were investigated. To develop an evidence-based system for analysing this, the following measurable objectives were evaluated:

1. To compare survivorship of Dalmatian toadflax between treatment methods;
2. To contrast treatment success and efficiency of treatment method;
3. To investigate the change within each life stage from baseline to post-treatment.

Methods

Site Description

This study was established in Kenna Cartwright Nature Park, Kamloops, British Columbia in 2016. The dominant vegetation type in healthy grassland communities of the park is characterized by bunchgrasses and forbs. This region experiences daily mean growing season temperatures between 5.2°C in March and late October to 21.5°C in July (ECCC 2016). Over this interval, monthly precipitation averages between 12.8 mm and 37.4mm (ECCC 2016). At approximately 800 hectares, Kenna Cartwright Nature Park is one of the largest urban parks in North America and experienced over 230,000 visits in 2016, of which 121,968 were through the nearest entrance relative to the study area (pers. comm. K Wourms) (Figure 1). Residents participate in hiking, mountain biking, snowshoeing and there are a significant number of off-leash dog walkers. The park is an important education tool through which approximately 300 students participate in annual tree planting, weed pulling and general nature programming. Bear, deer, coyote, small mammals, raptors and owls utilize habitat within the park (pers. comm. K Wourms). Due, in part, to the diversity within the protected area, there are multiple on-going and historic studies (pers. comm. K Wourms).

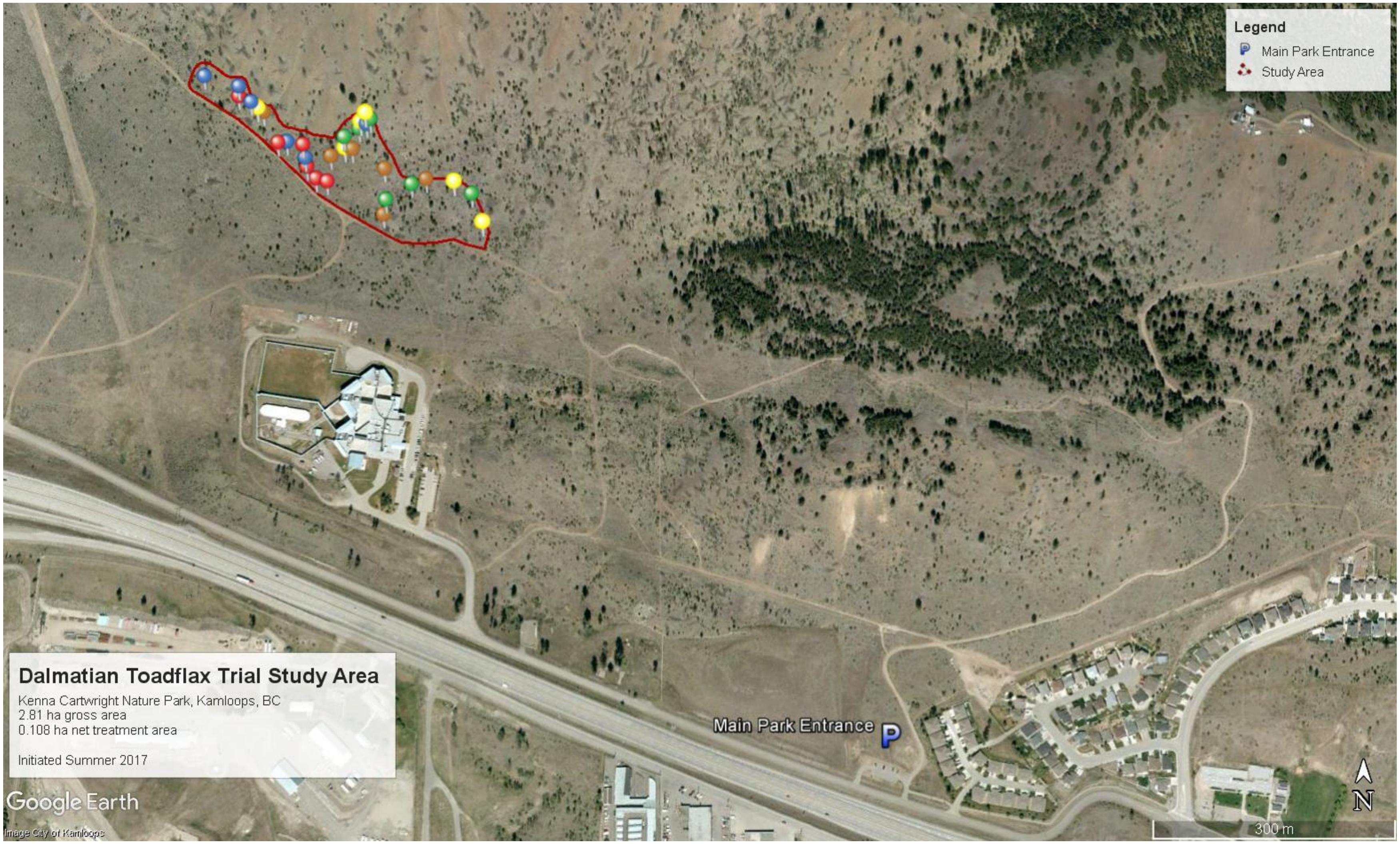

Figure 1. Study location in Kenna Cartwright Nature Park, Kamloops, British Columbia.

The primary recent ecosystem management tool in the Park has been prescribed fire and this study is situated in the most recent burn area. In 2016, 14.3 hectares were burned between March 14 to 23. Approximately 65-75% of this area experienced low intensity fire (Morrow 2016a). The study is situated in the western half of the burn area (pers. comm. K Wourms). Prior to the prescribed fire there was a fuel management project, during which pine was bucked, piled and burned from January 18 to 22 (Morrow 2016b). This 8.7 ha area did not include any of the study site. Other historic management activities include helicopter spraying with *Bacillus thuiringiensis* var. *kurstaki* for Douglas fir tussock moth management, goat grazing for noxious weed management, biocontrol release for invasive plant control and danger tree falling following the 2006 – 2008 mountain and western pine beetle epidemics (pers. comm. K Wourms).

Goats were used as a biological control between 2012 and 2015 for 10 days per year, typically in June, and the target for seed head consumption was set at 90%. During 2014 and 2015, the goat grazing was concentrated on the area where this study is now established. This control method was terminated in 2016 due to lack of monitoring and understanding of true success. Anecdotal evidence suggested that the goats were not decreasing the amount of target noxious weeds, such as Dalmatian toadflax, for which they were intended. Biological releases are continually made in the park targeting knapweed and Dalmatian toadflax using a variety of agents such as *Larinus spp.*, *Cyphocleonus achates* and *Mecinus janthinus.* On-going trail maintenance and facility improvement projects occur using foot crews, vehicles and heavy machinery.

In the 2002 Park Restoration Plan, herbicide treatment was not recommended for toadflax due to both the high chemical concentration levels required as well as the fact that M. janthinus biological control was effective at that time in eliminating large infestations of toadflax (Tarasoff 2002). Detailed information on the number of actions taken in response to these recommendations is not known. In 2002, there were 3.75 ha, 5.57 ha and 13.84 of light, moderate and heavy toadflax densities, respectively (Table 1). Additionally, there was a total area of 164.29 ha of Dalmatian toadflax mixed with knapweed, in varying compositions (Tarasoff 2002). The size of toadflax infestations throughout the park ranged drastically from 0.02 ha to 12.02 ha and when found with knapweed, the infestations ranged from 0.02 ha to 68 ha.   Biocontrol release, manual removal of sparse weeds and revegetation with native grasses was recommended for all toadflax composition and densities. Mechanical treatment (weed wack) of trails was advised in specific situations.

Table 1. Historic Kenna Cartwright Restoration Plan Dalmatian toadflax inventory and management recommendations. From Tarasoff (2002).

Dalmatian Toadflax Inventory			**Management Recommendations**			
Description	Infestations/ Total Area	Average Size and Range (ha)	Biocontrol sites >0.1 ha	Hand pull	Weed Wack Trails	Revegetate with native grasses
Light Toadflax	18/3.75 ha	0.21 (0.02-1.4)	10 releases needed	Yes	No	Yes
Moderate Toadflax	21/5.57 ha .	0.27 (0.02-0.97)	12 released needed	Yes	Yes	Yes
Heavy Toadflax	4/13.84 ha	3.36 (0.24-12.02)	5 releases needed	Yes	Yes	Yes
Light toadflax w/ Light knapweed	3/36.19 ha	12.06 (0.94-31.36)	6 toadflax releases needed	Yes	No	Yes
Light toadflax w/ Mod knapweed	3/2.29 ha	0.76 (0.04-1.34)	2 toadflax releases needed	Yes	Yes	yes
Light toadflax w/ Heavy knapweed	5/37.63 ha	7.53 (0.51-25.4)	8 toadflax releases needed	Yes	Yes	Yes
Mod toadflax w/ Mod knapweed	7/20.18 ha	3.01 (0.06-8.9)	8 toadflax releases needed	Yes	Yes	Yes
Heavy toadflax w/ Light knapweed	1/68.0 ha	68.0	8 toadflax releases needed	Yes	Yes	Yes

Baseline Sampling

Mean Dalmatian toadflax density was consistent across the study area ($p = 0.056$). On average, toadflax density is in the upper range of a medium density classification ($\bar{x} = 26$ m^2) according to the limited assessments of this kind available in the literature (Dodge et al. 2008). For the purpose of this study, stem count density followed the same categories as Dodge et al. (2008) and are used to explain site-specific management implications (Table 2).

Table 2. Dalmatian toadflax stem density categories per m^2, based on Dodge et al. (2008).

Category	Toadflax Stem Density (m^2)
Low	1-10
Medium	11-29
High	≥ 30

Plant community data was collected at baseline sampling but is not considered in this research. An import graminoid species within the area is *Pseudoroegneria spicata* which provides critical forage and wildlife habitat opportunities (Johnston 2008). Other grasses found onsite include *Hesperostipa comata*, *Koeleria macrantha* and *Poa secunda*. The forb community consists of primarily *Achillea millefolium*, *Goodyera oblongifolia*, *Antennaria spp.* and *Calochortus macrocarpus*. In addition to Dalmatian toadflax, alien species within the area include *Centaurea maculosa*, *Aruncus dioicus* and *Poa pratensis*. Although *Bromus tectorum* is found within 350 m of the study area, none was detected in field surveys. *Artemisia tridentata* and *Ericameria nauseosa* are scattered throughout the study area; however, plot stratification reduced the presence of shrubs to approximately zero to maintain consistency and to reduce the likelihood of mortality to these species via herbicide. *Pinus contorta* poles and veteran *Pinus ponderosa* are scattered throughout the hillside and were avoided in plot layout (Figure 2). The study area has a mean slope of 20% and south-western aspect. Gross study area is approximately 2.81 ha and total net treatment area is 0.108 ha. The site is boarded by a two-track road to the south and a single trail intersects the study area in the north western portion.

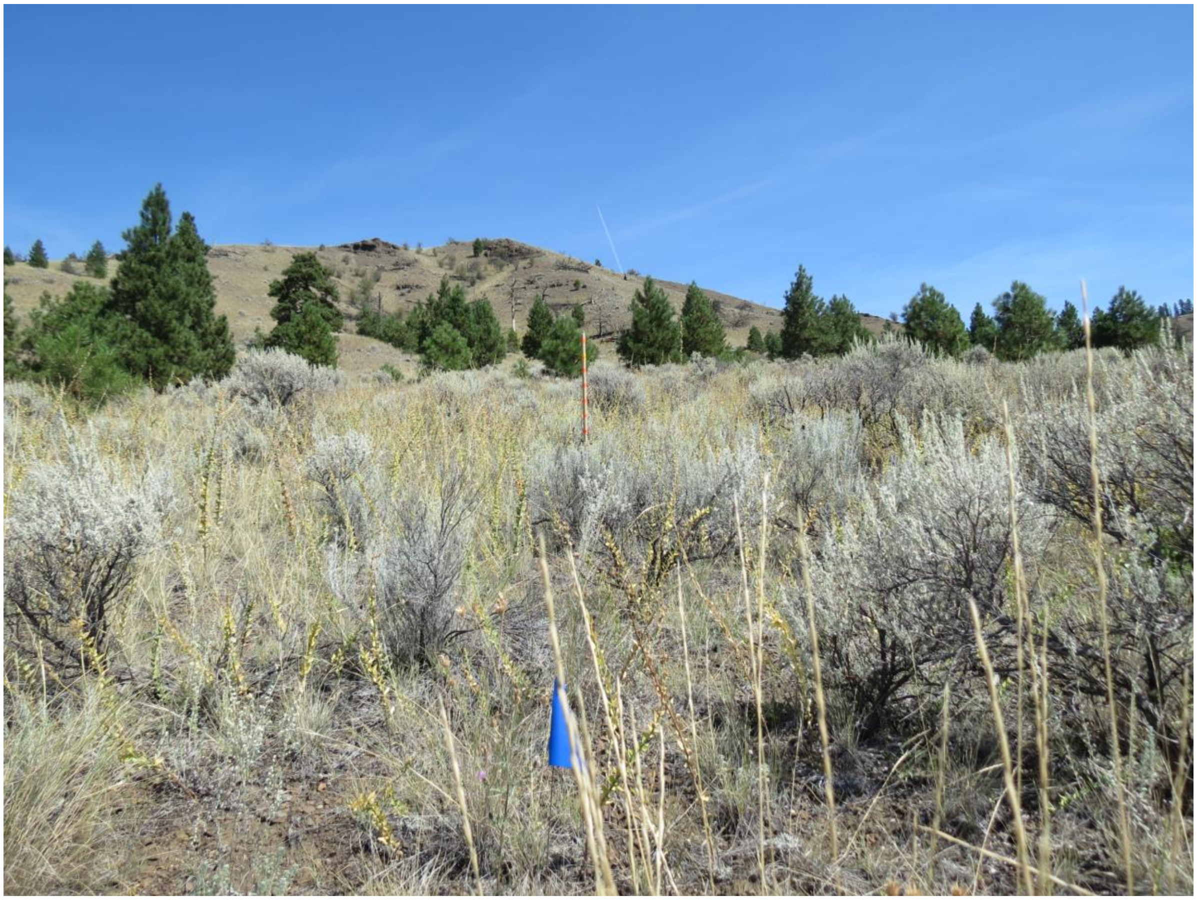

Figure 2. Representative photograph of Dalmatian toadflax invasion within study area in Kenna Cartwright Nature Park, Kamloops, B.C.

Experimental Design

Treatments

Manual and chemical treatments were implemented in an effort to identify the most successful and efficient control method for Dalmatian toadflax in this region. Manual removal was performed by hand pulling the plant and chemical treatments included broadcast and spot spraying using a backpack sprayer, as well as herbicide application through wicking (Figure 3).

a b

Figure 3. Herbicide application methods of broadcast and spot spraying via backpack sprayer (a) and hand wick (b).

Three chemical treatments were applied on August 23, 2017 during the early senesces life stage. The label recommends application when plants are actively growing through full bloom at a rate of 90 mL in 18 L solution per m^2 (DowAS 2009). Considering the seminal research and these regulatory limits, application rate was set at 213 g a.e. ha^{-1} picloram (Table 3). Non-ionic surfactant Agral 90 was added to all chemical solutions at a rate of 0.25% v/v to increase adhesion (SCI 2010). The manual removal concentrated efforts on pulling the greatest amount of root biomass from the soil as possible when soils were slightly moist and warm. All treatments were replicated six times and these plots were 6 m by 6 m.

Table 3. Treatment methods and type, chemical application rate and active ingredient concentration.

Treatment	Commercial Product	Application Rate	Tordon 22K A.E. Concentration	Actual A.E. Application Rate
Control	-	-	-	-
Manual Removal	-	-	-	-
Broadcast Spraying	Tordon 22K	8.8 L ha^{-1}	Picloram 240 g a.e. L^{-1}	213 g a.e. ha^{-1}
Spot spraying				
Wicking	Agral 90	0.25% v/v		

Broadcast application was calibrated by determining the rate of movement required to spray each plot with 762 mL of solution (approximately three minutes). The precision of this method was ± 20 ml remaining at the end of all replicates. The total solution used and time taken for all herbicide treatments was recorded, as well as the duration for manual removal. Volume of solution used in the wick treatment could not be precisely recorded due to the design of the hand applicator. It was, however, approximated using a graduated cylinder.

Plot Description and Design

Treatments were randomly assigned to pre-established plots (Figure 4). Two of the treatments (broadcast spraying and wicking) appear clumped but were not reassigned due to the uniformity of vegetation community, aspect and slope across the study area, as described previously in *Site Description.* Permeant sample points (PSP) are located in the bottom left corner of each plot (when facing north-east) and marked with a metal stake.

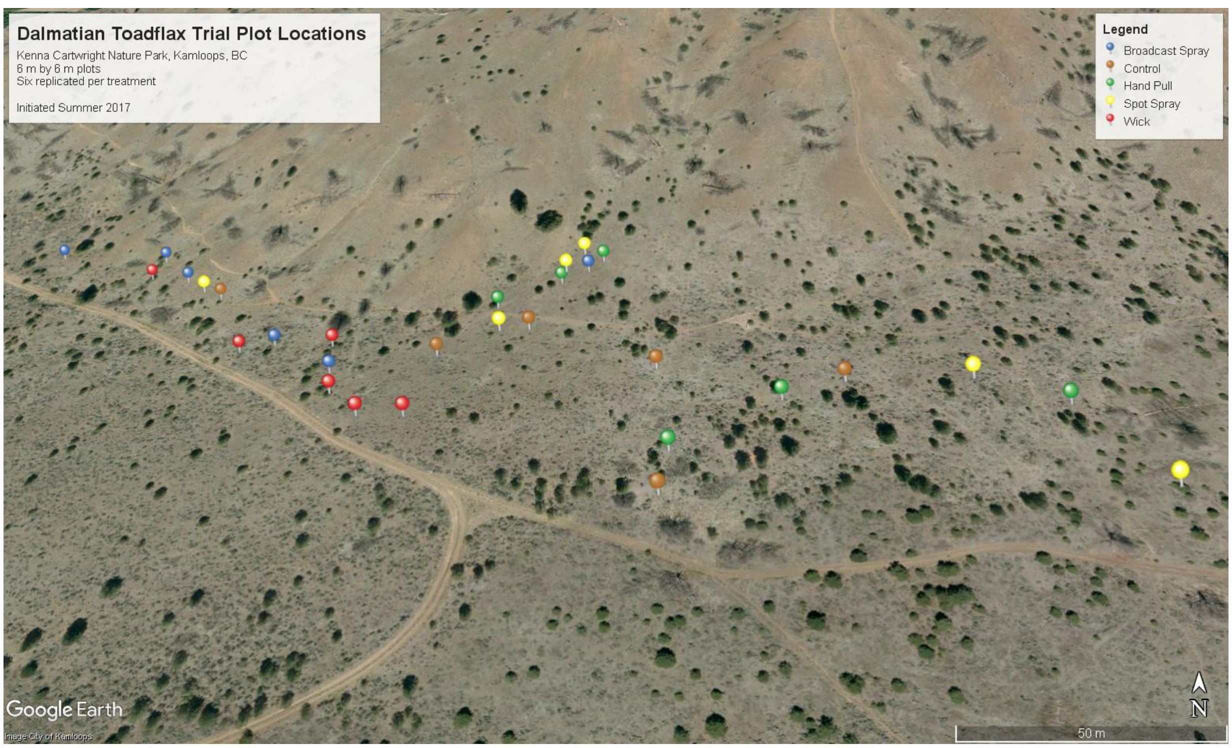

Figure 4. Study plot locations with treatment method and gross study area.

Each PSP was recorded in Universal Transverse Mercator coordinate system using the World Geodetic System (1984) projection. Azimuths of the “bottom” (to looker’s right) edge and left edge of the plot square were recorded from the PSP to allow for re-establishment (Appendix B: Plot Data).

There is a one meter sampling "buffer" around the margins of the plot resulting in an inner 4 m by 4 m square available for sampling (Figure 5). This inner area represents 16 m2 of the total 36 m^2 area. Toadflax density sampling was conducted in five 1 m^2 subplots per replicate at three sampling periods, one-week pre-treatment (August 17, 2016), two weeks post treatment (September 12, 2016) and end of growing season (October 22, 2017). The five subplots were randomly selected, with removal, from the available 16 and established using a 1 m by 1 m plot square as a measuring tool. This was completed at each sampling period and so, individual plots were not sampled through time.

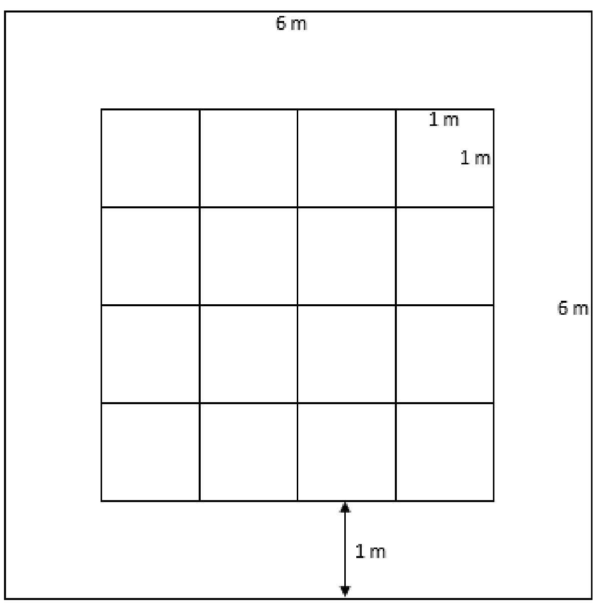

Figure 5. Plot design with sampling stratification method. Six replicates of each treatment (N = 30).

Field Data Collection

Count of toadflax stems in one of seven growth stage classifications was collected in the field and subsequently reclassified into vegetative, mature or dead after further literature review and statistical consideration (Table 4). Critical vegetation and site observations were recorded.

Table 4. Classification of Dalmatian toadflax life stage according to field assessment category.

Classification	Field Assessment Category
Vegetative	Seedling Rosette New stem growth (creeping)
Mature	Single stem flowerless Single stem flowers present Branched stem flowers present
Dead	Dead

Herbicide effectiveness was determined by surveying the toadflax basal region for living buds in addition to the above-ground evidence of herbicide uptake such as epinasty, callus tissue formation, leaf malformations and flag leaves (Gunsolus et al. 1999). The end-of-growing-season assessment was completed in the same manner to investigate residual mortality of toadflax, as well as the amount of regrowth within each treatment. Rosettes were an important metric at this sampling stage considering the vigorous root sprouting that toadflax is capable of. Each subplot was inspected for evidence of *Mecinus janthinus* during all sampling periods by looking for ovipositor and emergence holes in the stem as well as the weevil larvae, pupae and adults. There was no evidence of *Mecinus janthinus* found in any of the subplots or in surveyed populations of Dalmatian toadflax nearby.

To determine the current plant community, sampling of cover-by-layer was performed (Appendix A: Plant Community Summary). The canopy-by-species sampling method was modified from Daubenmire (1958) such that three Daubenmire rectangles were randomly assigned to one of the sampling corners and the actual cover (percent) was used instead of the cover classes outlined by Daubenmire. Bare exposed mineral soil and litter cover were recorded as separate layers. The cover sampling method was completed using a top-down analysis of the absolute cover of vegetative species, woody material, bare ground, feces, rock, and cryptogrammic crust.

Environmental data recorded for each site included weather, bearing, slope, aspect, elevation, and site descriptors. One photo was taken at each permanent sample point and followed the methodology described in Chapter 6 of the Grassland Monitoring Manual for B.C. to capture local conditions (Delesalle et al. 2009). The photographs were taken from two meters behind the permanent sample point and captured all four flagged corners, when possible.

Statistical Analysis

Mean living toadflax by treatment within each life stage (vegetative, mature and total-live) was used to investigate the treatment success. The analysis of variance between each treatment method and the control within the total live category dictates overall success. Investigation into the difference in means between vegetative and mature life stages offers further insight into appropriate timing of application.

Two sample groups were marginally non-normal (Shapiro-Wilk's test). Further investigation analysing normality quantile-quantile plots demonstrated no need to correct the normality. More important, though, was the failure to pass Levene's test of homogeneity of variances in five of nine groups. The residual scatter plots did not demonstrate a clear suitability for transformation and attempts to implement natural logarithm and square root transformations were unsuccessful.

Considering these limitations, a one-way analysis of variance was used when appropriate (mature life stage) and a Welch robust test of equality of means was used for non-homogeneous variances (total-live and vegetative life stages). The Welch ANOVA is effective for reducing the probability of Type I error when analysing data with great heteroscedasticity (McDonald no date). I performed a post-hoc analysis of the one-way ANOVA using Tukey-HSD. For the Welch ANOVA, I used a Games-Howell post hoc for a portion of vegetative and all of total-live life stages. Since the broadcast spray treatment was constant at zero stems per m^2 in the vegetative life stage, this variable was not suitable for inclusion in the analysis of variance since the standard deviation is zero. Instead, the remaining treatments were compared to zero using a one-sample t test (i.e. test value set to zero). This allowed for post-hoc comparison between broadcast spraying and all other treatments without jeopardizing the reliability of the analysis of variance between the remaining treatments.

To investigate whether there were significant differences between the baseline sampling and end-of-season count data within each life stage by treatment, I used paired t-tests for mature and total-live data. If data indicated non-homogenous variance I used related-samples Wilcoxon signed rank tests.

All test significance levels were set to $\alpha = 0.05$, two-tailed. All Welch ANOVA's were asymptotically F distributed. Visual investigation of normality assumption was completed in R version 1.22 using DAAG package. All statistical tests were performed in IBM SPSS Statistics for Windows, Version 24.0.

Results

Treatment Success

Treatment had a significant effect on end-of-season mean total-live stem count (Table 5). Further investigation into the aggregated data (by splitting vegetative and mature life stages) offers increased insight into the effect of treatments on specific life stages. At this level, there was a statistically significant difference in the vegetative life stage between treatments and not the mature life stage (Table 5).

Table 5. Analysis of variance within life stage between treatments for end-of-season Dalmatian toadflax mean live stem count. Asterisk identifies where Welch robust tests of equality of means were performed.

Life Stage	Treatment	N	D.F. Between; Within	F	p-value
Total Live*	Control	6	4; 11.031	24.134	<0.001
	Manual Removal	6			
	Broadcast	6			
	Spot Spray	6			
	Wick	6			
Vegetative*	Control	6	3; 10.189	14.947	<0.001
	Manual Removal	6			
	Spot Spray	6			
	Wick	6			
	Broadcast[1]				*See* Table 6
Mature	Control	6	4; 25	1.015	0.418
	Manual Removal	6			
	Broadcast	6			
	Spot Spray	6			
	Wick	6			

[1] Welch robust test of equality of means used for control, manual removal, spot spray and wick treatments. One sample t test used to compare broadcast spraying to all other treatments.

Table 6 summarizes the post hoc analysis method used for determining statistical difference between broadcast and all other treatments within the vegetative life stage.

Table 6. One-sample t-test summary used for post hoc comparison between broadcast spraying all other treatments. Test statistic set to zero (equal to broadcast standard deviation).

Treatment	T Statistic	D.F.	p-value (2-tailed)
Control	8.844	5	<0.001
Manual removal	4.635	5	0.006
Spot Spray	2.699	5	0.043
Wick	6.781	5	0.001

The amount of increase or decrease in density between baseline and end-of-season stem count data showed a significant treatment effect for the total-live and vegetative categories and not within the mature life stage (Table 7).

Table 7. Analysis of variance within life stage between treatments on the difference between baseline and end-of-season Dalmatian toadflax mean live stem count. Asterisk identifies where Welch robust test of equality of means was performed instead of one-way ANOVA.

Life Stage	Treatment	N	D.F. Between; Within	F	p-value
Total Live	Control	6	4; 25	12.050	<0.001
	Manual Removal	6			
	Broadcast	6			
	Spot Spray	6			
	Wick	6			
Vegetative*	Control	6	4; 12.022	23.783	<0.001
	Manual Removal	6			
	Broadcast	6			
	Spot Spray	6			
	Wick	6			
Mature	Control	6	4; 25	3.098	0.034
	Manual Removal	6			
	Broadcast	6			
	Spot Spray	6			
	Wick	6			

Total-live stem count post-hoc analysis identifies statistical similarity between broadcast and spot spraying ($p=0.309$), both of which had the highest overall success (Figure 6). Broadcast spraying is different from all other treatments except spot spraying, whereas spot spraying is also similar to manual removal ($p = 0.1$) and wicking ($p = 0.588$) treatments. The wicking treatment is statistically different from the broadcast treatment ($p = 0.004$) and control ($p = 0.007$), and similar to manual removal ($p = 0.201$). The control is only statistically similar to the manual removal treatment ($p = 0.843$). Most of these changes are evident by the post-treatment sampling period indicating a quick treatment effect and no real change in regrowth between this time and the end of the growing season. The vegetative life stage mirrors the total-live except that broadcast spraying is different from all treatments in this life stage, including spot spraying ($p = 0.043$) and spot spraying is different from manual removal ($p = 0.048$).

Overall, only the control increased in total-live stem count by the end of the growing season and this increase was not statistically different from its pre-treatment density. The broadcast and spot spraying treatments offer the greatest reduction in density, relative to baseline, and these are statistically different from all other treatments ($p < 0.001$). The broadcast and spot spraying were the only treatments to reduce vegetative stem count from the pre-treatment to end-of-season assessments. However, the broadcast treatment alone is statistically different from its pre-treatment density ($p=0.027$) (Figure 6; see asterisks). All treatments experienced significant reductions in mean stem count within the mature life stage. The hand pulling treatment removed all stems and it is clear that the only living stems at the end of the season are new growth since treatment. The number of dead stems in the post treatment sampling is greater than in the end of season assessment and can likely be attributed to variability within the sampling with removal methodology – see *Future Research* for details.

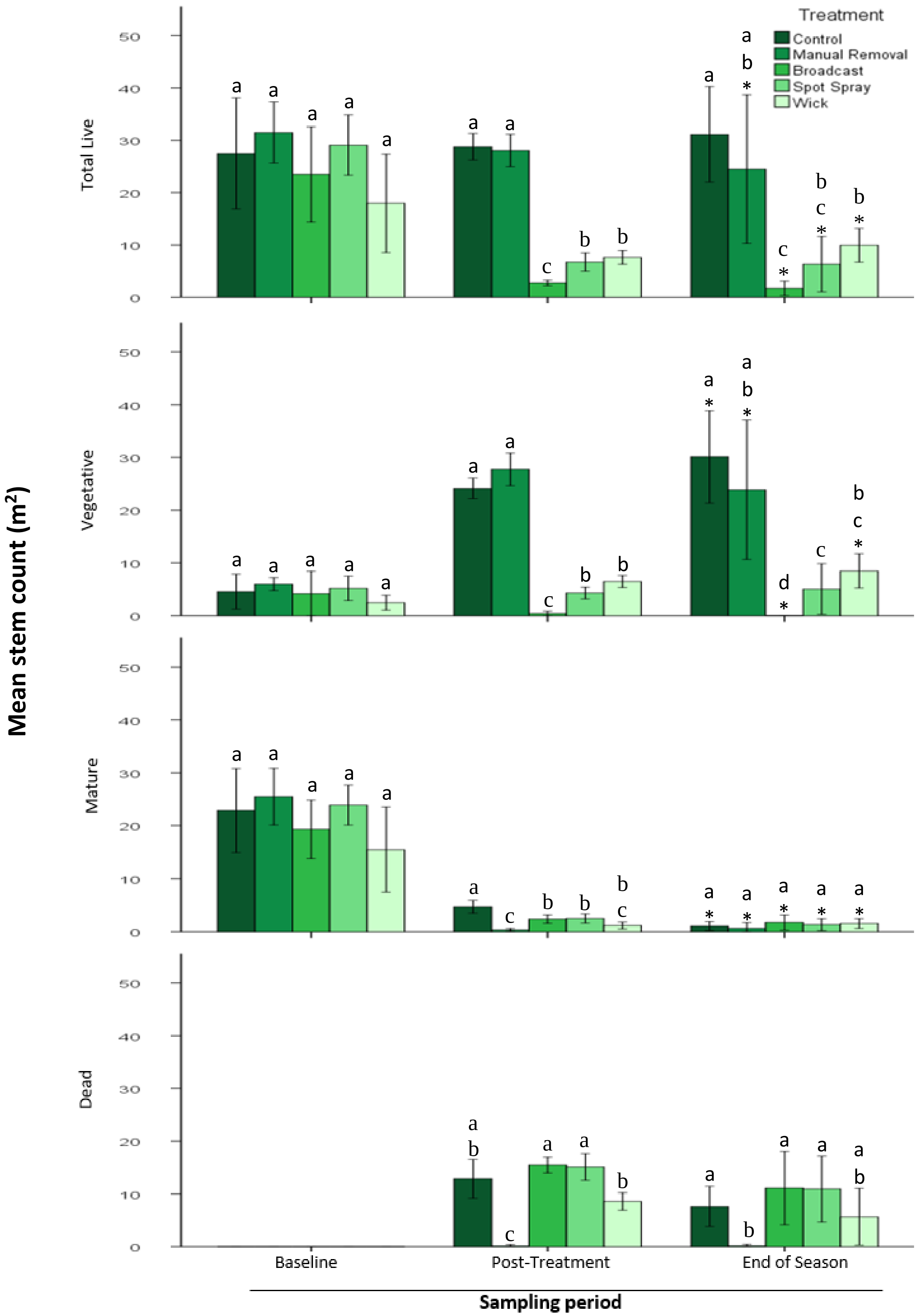

Figure 6. Mean stem count (m^{-2}) of total-live, vegetative, mature and dead life stages at baseline, post-treatment and end of growing season. Letters denote similarity within each life stage within each sampling period. Asterisks identify where the end-of-season live stem count is statistically different from its baseline.

The vegetative category encompassed seedlings, rosettes and new growth (creeping) (Table 3). Of interest is the change in seedling versus rosette density which is displayed in Figure 7. Seedling density remained relatively constant. However, the rosette density increased greatly in the control and manual removal treatments and marginally in the spot spray and wicking treatments by the end of the growing season. In the broadcast, there were no seedlings or rosettes detected during the final assessment. As a result of this, broadcast is only statistically similar to spot spraying in both of these life stages. Similar to the trend observed in total-live, manual removal is statistically similar to the control and different from all other treatments. In the rosette life stage, all chemical treatments appear to be different from the control and manual removal.

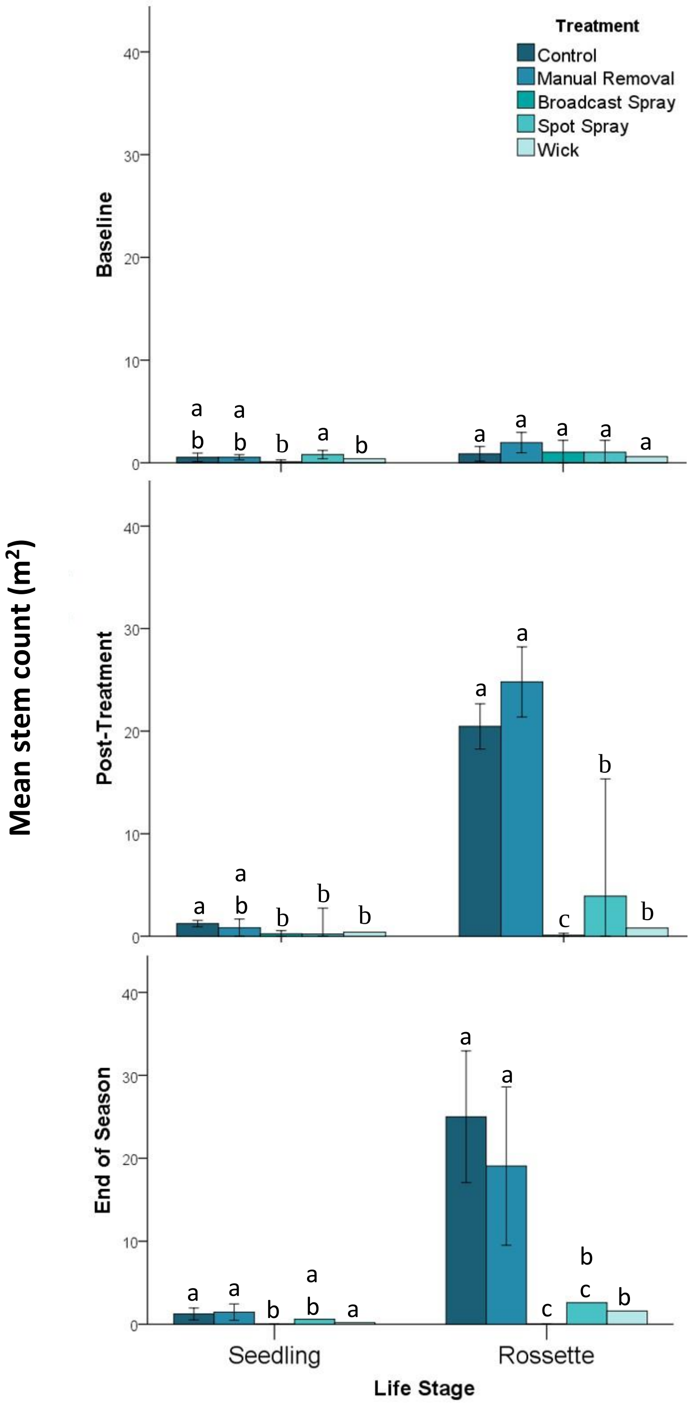

Figure 7. Seedling and rosette mean stem count (m^{-2}) at baseline, post-treatment and end of season assessments. Letters denote statistical similarity at the end of the growing season. Error bars are 95% C.I.

Treatment Efficiency

The duration of treatments varied greatly. Manual removal plots took 28 minutes longer on average than the longest herbicide treatment. Contrarily, the differences in duration of the three herbicide application methods were not as drastic (Table 8). Wicking was the slowest, followed by spot spraying and finally broadcast spraying.

Table 8. Average total solution volume, herbicide content, active ingredient concentration and treatment duration applied to replicates.

Method	Duration (min.)	Solution (mL)	Tordon 22K (mL)	Picloram (g a.e. replicate^{-1})
Control	-	-	-	-
Manual Removal	32 ± 7	-	-	-
Broadcast	3	762	32	7.8
Spot Spray	3.5 ± 1.5	567 ± 316	26 ± 16	6.4 ± 3.9
Wick	4.3 ± 1	371 ± 170	16 ± 6	3.8 ± 1.4

Broadcast spraying used the greatest volume of herbicide solution, on average. The wick method used half the amount of herbicide solution as broadcast spraying. Spot spraying had a large amount of variability, which was related to toadflax density (Table 9). Above 31 stems per m^2 (high density sites), spot spraying used more herbicide than broadcast spraying; this was the case in 50% of the replicates. Due to this variability, the actual picloram concentration in broadcast and spot spraying replicates was relatively close, compared to the wick application method.

The average reduction in stem count was 21 in the broadcast application and 20 in the spot spraying (Table 9). However, broadcast spraying had only 13% of the live stem density at the end of the growing season relative to baseline and spot spraying had 31%. Wicking replicates contained an average of 61% of the baseline density and had a mean reduction of 7 stems per m^2.

Table 9. Herbicide application method efficiency summary highlighting herbicide solution applied to each replicate and the baseline, post-treatment and end of growing season mean total-live stem count (m^{-2}).

Treatment	**Herbicide Solution (mL)**	**Baseline**	**Post-Treatment**	**End of Season**	**Difference***
Broadcast Spraying	762	38	3	1	-37
	762	22	3	5	-17
	762	26	3	4	-22
	762	18	3	5	-13
	762	12	2	1	-11
	762	25	2	3	-22
Average	762	24	3	3	-21
Spot Spraying	250	23	8	3	-20
	780	37	6	8	-29
	880	32	7	18	-14
	700	30	9	6	-24
	800	30	6	15	-15
	400	23	4	5	-18
Average	635	29	7	9	-20
Wick Application	250	16	8	10	-5
	450	28	6	17	-11
	375	29	7	11	-18
	450	7	9	9	2
	200	10	8	10	0
	500	19	9	11	-8
Average	371	18	8	11	-7

* Difference = (end of season – baseline) to represent survivorship rather than mortality.

Discussion

Evaluating Treatment Success and Efficiency

The objective of this research was to identify the most successful treatment method for removal of Dalmatian toadflax and contrast these results with application efficiency. There are several key trends in the response of Dalmatian toadflax to treatment methods that offer new understanding of the eradication possibilities for this invasive alien species. When selecting an appropriate control method, the broad-scale differences between manual removal and chemical treatment present the first insight into toadflax growth suppression.

Considering the similarity to the control in all analyses and long treatment duration, manual removal of Dalmatian toadflax offers little chance of successful eradication. When compared to the success of the chemical options, especially broadcast spraying, the manual removal is not a feasible or reliable method, which is consistent with previous studies (Scott 1999; Sheley and Clark 1999; Zouhar 2003; Kyser and DiTomaso 2013). Since hand pulling removed all stems, there was a decrease in the total-live stem count at the end of the growing season, relative to baseline. However, this must be contrasted with reproduction experienced by October of the same year. The increase in rosettes under this treatment method at both the post-treatment and end of season assessments suggests that it did in fact promote adventitious buds and has the potential to greatly influence the amount of growth in proceeding years. Further to this point we must consider the morphology of Dalmatian toadflax and how this influences the environmental impact of hand pulling; the extensive root system results is a great deal of soil disturbance. Therefore, while manual removal may remove current toadflax biomass, the exposed mineral soil following this treatment is highly susceptible to seedling establishment (Robocker 1970; Jacobs 2006). In an area occupied by various invasive and agronomic plants, this type of disturbance has the potential to promote further invasion and have a negative impact on the native ecosystem into the future (Clark 2003; Lajeunesse 1999 as cited in Kyser and DiTomaso 2013).

Despite the variety of chemical timing and application rates identified in the literature, very few studies have been able to truly evaluate the interaction between these. Adding to this lack of clarity is the fact that seminal research by Robocker (1968) applied incredibly high rates of picloram in (1.68 kg a.e. ha^{-1}), where more recent studies are within the 180-260 g a.e. ha^{-1} range (Ogden and Renz 2006; Jacobs 2006; DiTomaso et al. 2013). Considering these limitations, this study intended to identify the most appropriate control method at a moderate application rate of 213 g a.e. ha^{-1} with a focus on application method.

Broadcast and spot spraying provided the highest overall success resulting in mean stem counts of 1.7 ± 1.17 and 6.3 ± 2.7 per m^2, respectively, in the total-live category at the end of the growing season. The application of herbicide via wicking produced similar results as the manual removal in both the total-live and vegetative life stages. Contrarily, spot spraying only had this similarity in the total-live category. Given the small population size required to select wicking over spot spraying, the overall impact on the plant communities through time will offer more insight into the appropriate use of these two methods. Furthermore, when considering the survivorship experiences in each treatment, there are more pertinent details exposed. Wicking offered the same overall success as spot spraying in reducing total live stems based on mean stem count. However, wicking contained 61% of the live stems at the end of the growing season, relative to baseline, whereas spot spraying only contained 31%. Furthermore, the lack of soil residual herbicide activity, an important component of chemical toadflax eradication, is not offered by the wick treatment (Jacobs 2006).

Spot spraying within the total-live category is similar to the broadcast spraying and may offer a potential control method on low to medium density sites, since it is only on high density sites that this method uses more herbicide than broadcast spraying. Additionally, only spot and broadcast spraying provide statistically significant reduction in vegetative stems from pre-treatment to end-of-season. The density and distribution of toadflax must be considered when selecting between spot and broadcast spraying. Since in three of six replicates spot spraying used more herbicide than broadcast, the true efficiency of spot treatment is difficult to justify when density surpasses 31 stems per m^2. The duration of this treatment can vary greatly and may negatively impact operational success and cause increased financial costs that counter balance the savings in herbicide. However, on low density sites with patchy distribution of toadflax, spot spraying may allow land managers to reduce the environmental impact of chemical treatment on native broadleaf species, relative to broadcast spraying (Clark 2003; Jacobs 2006). Considering the intentions of the majority of land managers and society at large, this transferred and possibly increased financial cost is worth the environmental benefits.

Broadcast spraying was the only treatment to eliminate vegetative growth and reproduction at the end of the growing season and nearly did so two weeks post-treatment. It is for this reason that on high density sites broadcast spraying is the most appropriate treatment method. Broadcast application also offers reliable delivery rates and a standardized duration which will increase operational efficiency. This treatment method was quickest for high density sites and had the lowest average duration when considering all sites. Additionally, broadcast spraying produced the greatest success by resulting in a survivorship rate of only 13%. Broadcast application of picloram offers one of the most important components of toadflax control, soil residual activity. When applied during stages of active growth, before waxy cuticle development, this method has the greatest chance of maximizing eradication success via herbicide (Jacobs and Sheley 2005; Sheley 2006; Robocker 1968; Ogden and Renz 2005; DiTomaso et al. 2013). This is important to consider when rosettes were absent from the broadcast spraying at the end of the growing season and nearly all of the regrowth experienced by the four other treatments were rosettes.

The statistical analysis of treatment success was subject to a large degree of variability in the manual removal, spot spraying, wick and control treatments. Only the broadcast spray had low standard deviation throughout the experiment. Despite how this potentially influences test results, clear (and often substantial) statistical differences were detected between treatments. Additionally, there was a clear trend throughout the life stages that chemical treatments were a further distance from manual removal and control than they were from each other. Future research should standardize sampling in a way that minimizes variability and then focus on tracking the plant communities through time.

Management Implications

This research highlights the importance of planning invasive plant management on small scales and provides land managers with multiple integrated management components to consider when removing Dalmatian toadflax from semi-arid grasslands:

1. On high density sites, broadcast spraying offers the greatest success as well as highest efficiency and predictability. It also removed all vegetative reproduction at the end of the growing season and offers soil residual activity;

2. On low to medium density sites spot spraying is an efficient and effective method. However, it does not eliminate vegetative reproduction by the end of the growing season which may impact future biomass accumulation;

3. Manual removal is not recommended as it is not statistically different from the control. It also has the potential to increase soil disturbance and appears to promote adventitious root buds;

4. Wicking is time consuming relative to other herbicide application methods and has the lowest chemical eradication success. Further research through time may lend more insight into the applicability of this method on low density sites where soil residual activity is not desired.

Future Research

The high variability within stem count data is a result of the sampling method and should be addressed before proceeding with future research. In herbicide efficacy studies, mortality is evaluated on a stem-by-stem basis and each individual plant is marked and studied. Since this was a field-based operational study, this strict level of stem count data at an entire plot level was not feasible and thus introduced variability into the data that may have been reduced using other sampling methods. It is recommended that a permeant area be marked for sampling and all stems within this area should be counted. Timing of application for this study was within the range of recommendations cited in the literature. However, an earlier application (pre-bolting) has recently shown to be successful and could be targeted for future management of Dalmatian toadflax (Kyser and DiTomaso 2013). Considering the high statistical evidence, timing of application is less important than the community-level impacts of treatments in future research.

The primary question going forward is how the plant community as a whole will respond to the treatment methods, which were solely researched for and targeted at Dalmatian toadflax control. Future research should take the baseline plant community data and track the response of individual species through at least the next two years. Given the selectivity of Tordon 22K, native forbs and other invasive species could respond in different ways to both the herbicide residual activity and the absence of Dalmatian toadflax. Additionally, soil information should be collected (specifically pH and texture) to evaluate the success of Tordon 22K relative to other studies when considering the potential residual activity.

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Appendix A: Plant Community Summary

Table 1. Study area plant community summary based on selectively placed plot locations.

Functional Group	Species	Cover (%)
Grasses	*Pseudoroegneria spicata*	21
	Hesperostipa comata	7
	Koeleria macrantha	2
	Poa secunda	6
Forbs	*Achillea millefolium*	2
	Goodyera oblongifolia	<1
	Antennaria spp.	2
	Calochortus macrocarpus	<1
	Agoseris glauca var. dasycephala	1
	Geum triflorum var. ciliatum	<1
Shrubs	*Artemisia tridentata*	2
	Ericameria nauseosa	1
Trees	*Pinus contorta*	<1
	Pinus ponderosa	<1
Invasive	*Linaria genistifolia ssp. dalmatica*	34
	Centaurea maculosa	4
	Aruncus dioicus	2
	Poa pratensis	2
	Grindelia squarrosa var. serrulata	1
Ground Cover (∑100%)	Bare Ground	13
	Rock	4
	Cryptogrammic Crust	63
	Litter	20

Appendix B: Plot Data

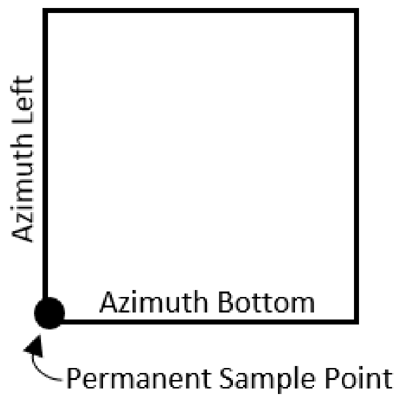

Figure 1. Plot re-establishment guide. Permanent sample point corresponds with UTM coordinates outlined in Table 1.

Table 1. Plot and treatment information summary including permanent sample point coordinates, block azimuths, treatment duration, herbicide application amount and volume of chemical applied.

Plot	Treatment	UTM Zone	Easting	Northing	Azimuth Bottom	Azimuth Left	Time (min.)	Solution Volume (mL)	Water (mL)	Tordon (mL)	Agral 90 (mL)
1	Broadcast Spray	10 U	682613.1	5616353	135	45	3	762	711.00	32.00	19.00
2	Wick	10 U	682660.3	5616339	50	140	4	250	233.64	10.51	5.84
3	Broadcast Spray	10 U	682659.9	5616328	48	138	3	762	711.00	32.00	19.00
4	Broadcast Spray	10 U	682676.5	5616320	132	42	3	762	711.00	32.00	19.00
5	Spot Spray	10 U	682685.3	5616313	114	24	2	800	747.66	33.64	18.69
6	Control	10 U	682724.2	5616271	142	52	-	-	-	-	-
7	Broadcast Spray	10 U	682693.2	5616308	130	40	3	762	711.00	32.00	19.00
8	Wick	10 U	682712.2	5616270	112	22	4.25	450	420.56	18.93	10.51
9	Wick	10 U	682745.5	5616268	118	28	4.5	375	350.47	15.77	8.76
10	Broadcast Spray	10 U	682748.9	5616251	120	30	3	762	711.00	32.00	19.00
11	Wick	10 U	682751.4	5616240	138	48	3.25	450	420.56	18.93	10.51
12	Wick	10 U	682764	5616225	178	88	5.25	200	186.92	8.41	4.67
13	Wick	10 U	682778.9	5616221	134	44	4	500	467.29	21.03	11.68
14	Control	10 U	682783.5	5616253	118	28	-	-	-	-	-
15	Spot Spray	10 U	682802.3	5616263	138	48	3.5	780	728.97	32.80	18.22
16	Hand Pull	10 U	682801.1	5616276	116	26	33	-	-	-	-
17	Control	10 U	682821.3	5616286	116	26	-	-	-	-	-
18	Hand Pull	10 U	682823.1	5616295	134	44	38	-	-	-	-
19	Spot Spray	10 U	682829.1	5616305	128	38	5	400	373.83	16.82	9.35
20	Spot Spray	10 U	682835.8	5616297	118	28	4.5	250	233.64	10.51	5.84
21	Hand Pull	10 U	682831	5616291	116	26	24	-	-	-	-
22	Broadcast Spray	10 U	682854.1	5616238	104	14	3	762	711.00	32.00	19.00
23	Control	10 U	682856.1	5616200	126	36	-	-	-	-	-
24	Hand Pull	10 U	682853.3	5616181	81	351	35	-	-	-	-
25	Control	10 U	682889.4	5616220	100	10	-	-	-	-	-
26	Hand Pull	10 U	682908.3	5616226	122	32	34	-	-	-	-
27	Control	10 U	682967.6	5616208	118	28	-	-	-	-	-
28	Spot Spray	10 U	682982.3	5616174	91	1	3	800	747.66	33.64	18.69
29	Hand Pull	10 U	682944.4	5616223	90	0	27	-	-	-	-
30	Spot Spray	10 U	682812.3	5616261	110	20	3	700	654.21	29.44	16.36